

# Fabrication inside Microchannels Using Fluid Flow

PAUL J. A. KENIS, RUSTEM F. ISMAGILOV,  
SHUICHI TAKAYAMA, AND  
GEORGE M. WHITESIDES\*

*Department of Chemistry and Chemical Biology,  
Harvard University, Cambridge, Massachusetts 02138*

SHULONG LI† AND HENRY S. WHITE\*

*Department of Chemistry, University of Utah,  
Salt Lake City, Utah 84112*

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## ABSTRACT

This Account summarizes techniques for carrying out microfabrication of structures with dimensions down to 10  $\mu\text{m}$  in microchannels that are 0.02–2 mm wide. These methods are largely based on the exploitation of laminar flow at low Reynolds number ( $Re$ ) to control the spatial delivery of reagents. These methods are illustrated by fabrication of fibers, microelectrode arrays, arrays of crystals, and patterns of proteins and cells.

## Introduction

Channels that are 0.02–2 mm wide—channels which, in this Account, we call almost interchangeably “microchan-

nels” or “capillaries”—can be considered as reaction vessels with two unusual characteristics. First, their interior volumes are small, but readily and rapidly accessible from the outside by pumping fluids or gases into them. Second, fluids moving in them at low to moderate velocities flow laminarily, that is, without turbulence. This laminar flow can be used to deliver reagents spatially inside capillaries with remarkable precision. These characteristics, combined with the ease with which microchannels can be assembled and disassembled using soft lithography and other techniques, suggest them as a new system of reactors with which to carry out microfabrication.<sup>1</sup>

This Account summarizes projects in our two laboratories that are aimed at developing techniques for microfabrication that use microchannels as reactors. We had two objectives. First, we wished to use the small volume of the microchannel to generate microstructures that would be removed once formed and used independently of the channel in which they were formed; that is, the microchannel would serve as a reactor for the fabrication of a structure to be used elsewhere. Second, we wished to fabricate small, functional structures inside capillaries, with the intention of leaving the structures in place once fabricated. These structures would be integrated with other uses of the channel (for example, as microelectrodes in a microanalytical system in which separations are carried out in the capillary). The techniques that are reviewed in this Account provide routes to structures that are difficult to fabricate by other techniques. These methods are especially useful in handling proteins, cells, and other delicate biological structures.

To accomplish either task, it is necessary to perform a set of elementary operations: (i) transfer reagents from reservoirs outside the channel to the inside; (ii) deliver these reagents to locations where reactions are to occur; (iii) allow or cause the desired reactions; and (iv) remove waste products from the regions where reaction takes place. We have used fluids to accomplish these operations. In principle, one could also work with gases, although their distinct physical properties—for example, high diffusion constants—make their application in fabrication inside microchannels more challenging than that of liquids.

We organize this Account into four sections. In the first, we give a brief overview of the technologies that have been used to carry out microfabrication in microchannels. In the second, we review the characteristics of liquid flow in capillaries at low and high values of the Reynolds number,  $Re$ . The emphasis in this section is on the potential of laminar or near-laminar flow as a strategy to control the spatial delivery of reagents inside small channels. This strategy can be adapted either to a single liquid stream, or to two or more different streams of liquid in laminar flow. In the third, we describe examples of microfabrica-

Paul J. A. Kenis was born in 1970 in Arcen, The Netherlands. He received his masters degree from Nijmegen University (NL) with R. J. M. Nolte in 1993 and his Ph.D. degree in supramolecular chemistry from the University of Twente (NL) with D. N. Reinhoudt in 1997, after which he joined the group of G. M. Whitesides at Harvard University as a postdoctoral fellow. He joined the faculty of the department of Chemical Engineering at the University of Illinois at Urbana-Champaign in the fall of 2000. His research interests include (organic) materials science, surface chemistry, microfluidics, and microfabrication.

Rustem F. Ismagilov was born in 1973 in Ufa, Russia. He received his undergraduate degree from the Higher Chemical College of the Russian Academy of Sciences in 1994 and his Ph.D. degree in chemistry from the University of Wisconsin, Madison, with S. F. Nelsen in 1998. Currently he is a postdoctoral fellow in the group of Professor G. M. Whitesides at Harvard University. His research interests include physical organic chemistry, microfluidics, and nonlinear dynamics.

Shuichi Takayama was born in 1968 in Tokyo, Japan. He received his masters degree from the University of Tokyo with K. Mori in 1994 and his Ph.D. degree in chemistry from the Scripps Research Institute with C.-H. Wong in 1998, after which he joined the Whitesides group as a Leukemia and Lymphoma Society postdoctoral fellow. He joined the faculty of the department of Biomedical Engineering at the University of Michigan, Ann Arbor, in the fall of 2000. His major research interests are in the areas of bioorganic chemistry, biomaterials, bioengineering, and microfluidics.

George M. Whitesides received his A.B. degree from Harvard College in 1960, and his Ph.D. from the California Institute of Technology with J. D. Roberts in 1964. He was a member of the faculty of the Massachusetts Institute of Technology from 1963 to 1982, at which time he joined the Department of Chemistry at Harvard University. His present research interests include micro- and nanofabrication, microfluids, self-assembly, rational drug design, and cell biology.

Shulong Li was born in 1964 in Sichuan, China. He received his B.S. degree in chemistry and M.S. degree in polymer science from the University of Science and Technology of China, and his Ph.D. in materials science from the University of Minnesota in 1993, working with Professor H. S. White. He spent one year as a postdoctoral research associate at the National Renewable Energy Laboratory in Golden, CO, before joining the Milliken Research Corp. in 1994. He is currently conducting industrial research in the area of textiles and fibers.

Henry S. White is a Professor of Chemistry at the University of Utah. He received his B.S. degree from the University of North Carolina in 1978, and his Ph.D. from the University of Texas at Austin with A. J. Bard in 1983. He held a postdoctoral research appointment at the Massachusetts Institute of Technology from 1983 to 1984, and was on the faculty of the Department of Chemical Engineering and Materials Science at the University of Minnesota between 1984 and 1993. His present research interests include electroanalytical chemistry, molecular transport at nanostructured electrodes, iontophoretic transport in biological membranes, and magnetic field effects in electrochemistry.

\* To whom correspondence should be addressed.

† Current address: Milliken Research Corp., Spartanburg, SC 29304.

**Table 1. Techniques Used for Microfabrication inside Microchannels**

Strategy for Microfabrication Process or Technology	Reference
<b>Multilayer Photolithography</b>	2
<b>Single-Stream Liquid Flow</b>	
Chemical Vapor Deposition	3
Light Tweezers assisted Deposition	4
Polymer Deposition	8
Electroless Deposition	9
Electrochemical Fiber Growth	10
Etching of Optical Fibers	11,12
Micromolding in capillaries (MIMIC)	13-17
Deposition of Proteins	18
<b>Multiple-Stream Liquid Flow</b>	
Electroless Deposition	20
Etching	20
Nucleation and Crystallization ( $\text{CaCO}_3$ )	20
Deposition of Proteins	24
Deposition of Cells	24

tion that use a single stream of liquid. In the fourth, wesketch applications of microfabrication (and closely related methods used for micropatterning of bacterial and mammalian cells) based on multiple streams of liquid in laminar flow.

## I. Overview of Microfabrication in Microchannels

Table 1 lists technologies (including our own) that have been used to carry out microfabrication in capillaries. Conventional photolithography is the basis of the methods most commonly used to fabricate microchannels containing functional microstructures.<sup>2</sup> In this type of fabrication, the capillary and its internal features are generated by a series of planar pattern-transfer steps. Until recently, there were few alternatives to photolithography (and the processes intimately associated with it: evaporation, CVD, RIE, lift-off, and others). Miyagi et al. have, for example, patterned the inner walls of hollow waveguides by chemical vapor deposition of dielectric films consisting of  $\text{Cu}_2\text{O}$ .<sup>3</sup> Renn and Pastel have shown that laser-induced forces (light tweezers) can be used to manipulate and position atoms, clusters, and micrometer-sized particles (e.g., NaCl and KI crystals) inside hollow optical fibers.<sup>4</sup>

## II. Laminar and Turbulent Flow of Liquids: The Reynolds Number

The Reynolds number,  $Re = \nu\rho/\mu$ , characterizes the tendency of a fluid to develop turbulence, or to flow without turbulence (that is, laminarly).<sup>5</sup> Here,  $\rho$  is the density in kilograms per cubic meter,  $\mu$  is the viscosity in kilograms per meter per second,  $l$  is the diameter of the capillary in meters, and  $\nu$  is the flow rate in meters per second. The value of  $Re$  at which flow changes from turbulent to laminar depends on the geometry of the channel. For a straight pipe, this transition occurs at  $Re \approx 2100$ . Flow past an object or flow in a channel in which



**FIGURE 1.** Optical micrograph of two-phase laminar flow: two aqueous phases, each colored with a different dye (black ink and Congo red), brought together with a "Y" junction in an elastomeric microfluidic system.

the geometry changes, however, develops eddies at lower Reynolds numbers ( $Re \approx 40-300$ ). Liquid flows in capillaries (that is, tubes having small values of  $l$ ;  $l \approx 50-100 \mu\text{m}$ ) often have low values of  $Re$  and are therefore laminar, even for liquid flow velocities as high as  $0.5 \text{ m s}^{-1}$ . Most usefully, two or more streams that are brought together in a narrow channel flow laminarly, in parallel, without turbulent mixing (Figure 1). The only mechanism of mixing of their components is diffusion across the interface between the streams.

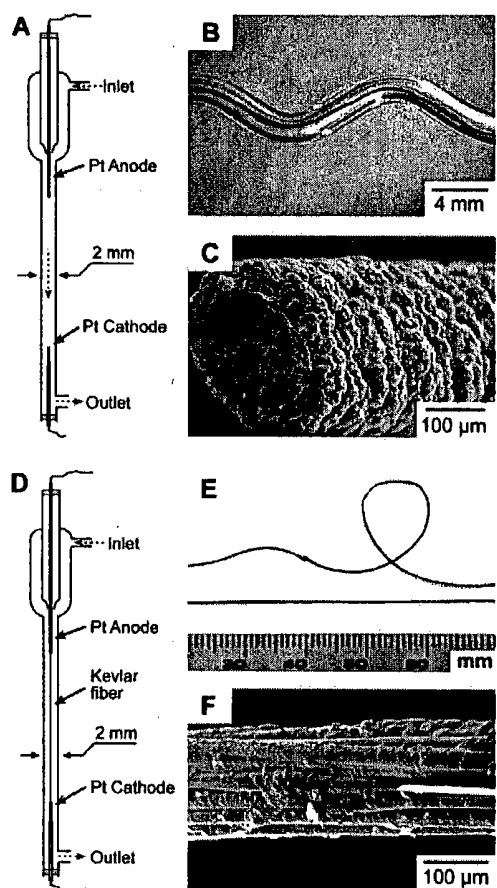
Laminar flow permits control of the spatial delivery of reagents within a capillary. The application of different chemistries from different streams of a multiphase, laminar flow system allows reactions to take place exclusively at certain regions of the capillary: either between a stream and the interior surface of the capillary, or at the interface between adjacent streams. Levin et al. pioneered the use of laminar flow in microfluidic devices to fractionate samples by taking advantage of the fast diffusion of small molecules or ions from one stream into an adjacent stream, relative to the slow diffusion of proteins, colloids, etc.<sup>6</sup> Weigl and Yager implemented these principles elegantly in a micromachined device for the analysis of heterogeneous biological samples such as blood by taking advantage of the fast diffusion of analyte molecules from the sample stream into an adjacent stream that contained, for example, a fluorescent indicator.<sup>7</sup>

In small capillaries, turbulent flow of aqueous solutions (or eddy-like flow in the region of transition from laminar to turbulent) occurs only at high velocities of flow. Turbulence facilitates mass transport. In laminar flow, the only mechanism for mixing is diffusion. This difference in mechanisms and rates of mass transport can be used to increase the rate of delivery of reagents to specific regions inside a capillary where flow is turbulent, and thus to increase the rate of chemical reaction in the turbulent regions, relative to regions where flow is laminar.

## III. Microfabrication Using Single Streams of Liquid in Microchannels

We distinguish different methods of fabrication inside microchannels using a single stream of liquid: one that results in a uniform modification of the inner surface of the capillary, and several others that allow for fabrication with spatial resolution.

**Uniform In-Channel Coatings.** A uniform coating—either covalently attached or physically adsorbed—on the inner surface of capillaries can easily be achieved from a single liquid stream—laminar or turbulent—that contains



**FIGURE 2.** (A) Schematic diagram of the electrochemical flow cell used to grow single-phase conducting polymer fibers. (B) Optical micrograph of a zigzag poly(3-methylthiophene) fiber grown in a zigzag glass capillary. (C) SEM image of a fracture cross section of a poly(3-methylthiophene) fiber. (D) Schematic diagram of the electrochemical flow cell used to grow composite Kevlar/conducting polymer fibers. (E) Optical micrograph and (F) SEM image of Kevlar/polypyrrole composite fibers.

the necessary chemicals. This strategy is frequently used to produce coatings: examples include organic films that reduce sticking of proteins in capillary electrophoresis capillaries<sup>8</sup> and metal films (by electroless deposition of silver) with polymer coatings inside hollow waveguides to reduce optical losses.<sup>9</sup>

**Conducting Polymer Fibers.** An example of fabrication from a single homogeneous stream that creates a free-standing microstructure is the electrochemical processing of conducting polymer fibers.<sup>10</sup> Figure 2A shows an electrochemical cell used to synthesize macroscopically long fibers of polypyrrole and poly(3-methylthiophene). Hydrodynamic flow patterns inside a 2-mm-diameter glass tube (separating the anode and cathode) are employed to control both the spatial direction and the rate of polymerization. A solution containing the monomer flows through the cell at an average velocity between 10 and 60  $\text{cm s}^{-1}$  (corresponding to  $700 < Re < 4200$ ). Nucleation and growth of a single conductive fiber from the anode in the direction of flow occur when an electrical current ( $\sim 1 \text{ mA}$ ) is passed between the electrodes. Controlling the

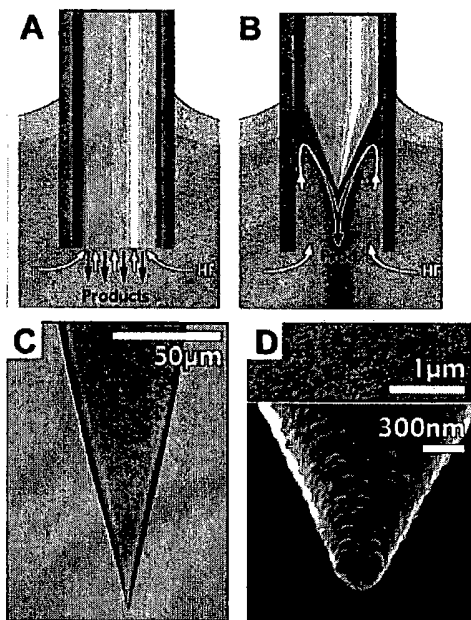
applied current or the flow velocity in the glass tube controls the rate of polymer growth as well as the shape of the resulting fiber: The application of larger currents typically resulted in faster growth of uniform fibers with larger diameters, whereas high flow rates ( $\sim 50 \text{ cm s}^{-1}$ ) resulted in nonuniform, cone-shaped fibers. Polypyrrole and poly(3-methylthiophene) fibers of 10 cm length and between 0.01 and 0.1 cm diameter have been grown using this one-step process (Figure 2B,C) with a wide variety of shapes (e.g., tapered fibers, zigzag-shaped fibers) by changing the shape of the glass tubing.

The key element of this procedure that gives directional polymer growth is the coupling of the flow patterns with mass transport of the monomer to the surface. Hydrodynamic flow past the tip of the growing fiber produces a relatively stagnant boundary layer or a recirculating flow in which intermediates produced by chemical reaction can be trapped. The result is an increased rate of deposition of polymerization intermediates (oligomers) at surfaces adjacent to the boundary layer or the fluid eddy, here the tip of the fiber. Consequently, the polymer fiber grows only along the direction of the flow within the glass tube, never touching the sides of the tube.

**Kevlar/Polypyrrole Composite Fibers.** Kevlar/polypyrrole composite fibers that exhibit excellent electrical conductivity and that are flexible and strong—in contrast with the brittle polypyrrole and poly(3-methylthiophene) fibers (see above) that break when flexed—have been synthesized in electrochemical flow cells using a different approach (Figure 2D).<sup>10</sup> A woven Kevlar thread, containing 70 filaments each of  $17 \mu\text{m}$  diameter, was suspended between the anode and cathode of the cell. When pyrrole was oxidized at the anode in the presence of flow ( $20 \text{ cm s}^{-1}$ ), the resulting polymer grew onto the Kevlar thread and coated the entire 10-cm length in  $\sim 20 \text{ min}$ . The conductive polymer phase deposits uniformly between the filaments of the Kevlar thread and produces an electrically conductive and flexible composite fiber (Figure 2E,F) that may be of interest for antistatic weaves and as flexible electrodes for *in vivo* measurements.

**Near-Field Optical Probes.** In a different strategy, Lambelet et al. and Stöckle et al. have used the film of polymer (the cladding layer) on the outside of optical fibers as capillaries with 3–200- $\mu\text{m}$  inside diameters, and then used convectively driven laminar flows inside these capillaries to fabricate high-quality, near-field optical probes with regular conical tips.<sup>11,12</sup> Hanging the ends of optical fibers with polymer cladding attached in an aqueous phase containing HF resulted in anisotropic etching of the glass cores, even without the application of bulk flow. The authors explain the formation of conical tips inside the cladding capillary by the combination of local flows induced by chemically driven convection and increases in the density of the solution on dissolution of the glass (Figure 3).

**Selective Patterning on Substrates.** We have demonstrated selective electroless deposition of metallic copper on one wall of a rectangular microchannel using a variation of the MIMIC (“micromolding in capillaries”)



**FIGURE 3.** Fabrication of near-field optical probes by tube etching as proposed by Stöckle et al. Schematic of the convection mechanism: (A) initial diffusion-controlled etching—more etchant available to the outer regions of the fiber—and (B) convection-controlled tip formation inside the tube driven by concentration gradients and the gravitational removal of reaction products. (C) Optical micrograph of an uncoated tip. (D) Scanning electron micrographs of an Al-coated tube-etched fiber tip. Top: the glass surface close to the tip apex. Bottom: the tip apex. Reproduced with permission from ref 12.

technique.<sup>13</sup> The channel consisted of a poly(dimethylsiloxane) (PDMS) membrane with a channel structure embossed in its surface placed on a gold-covered substrate. The channel was filled with an electroless deposition solution containing copper ions and formaldehyde as the reducing agent. The activity of the gold surface in catalyzing the electroless deposition process resulted in selective deposition of copper on the wall of the channel formed by the gold surface and not on the walls of the channel formed by the PDMS mold.

Similarly, Biebuyck et al. patterned regions of a substrate with immunoglobulins from a single liquid flow in a microfluidic network embossed in PDMS that was placed on the substrate.<sup>14</sup> This PDMS mold was passivated with poly(ethylene glycol) or bovine serum albumin prior to placement on the substrate to avoid deposition of immunoglobulins on its surface.

**Arrays of Beads.** A related technique also gives access to crystalline arrays of 0.1–3- $\mu\text{m}$  beads inside 10–100- $\mu\text{m}$  channels. The liquid flow (driven by evaporation from one end of the capillary, or by capillary filling of the tube) delivered the beads from suspension to a region in which they are, in essence, filtered from the flowing stream; this flow led to crystalline arrays. Harrison et al. and Manz et al. have used such arrays as packed reactor beds in microanalytical devices.<sup>15</sup> Xia et al. have exploited related flow behavior between narrowly spaced plates to fabricate crystalline arrays of beads; these crystals may be useful

as macroporous membranes,<sup>16</sup> tunable optical filters,<sup>17</sup> and 3D photonic band gap materials.<sup>18</sup>

These studies established the concept of using the capillary as a small reactor, and identified some of the types of chemistry that can be carried out using this type of reactor. The range of structures that one can fabricate inside microchannels using only a single stream of liquid is, however, limited.

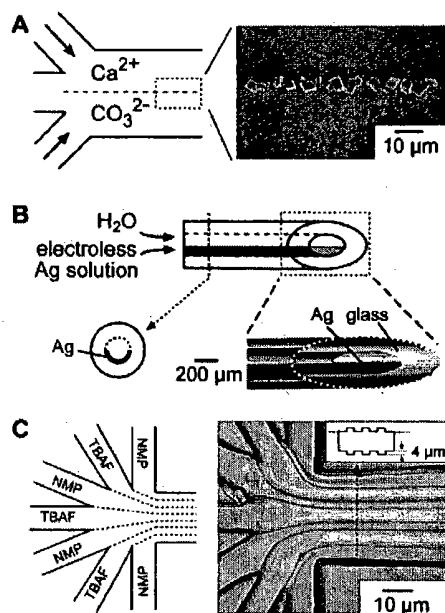
#### IV. Microfabrication Using Multiple Streams of Liquid in Laminar Flow

Soft lithography allows the fabrication of branching capillary networks rapidly, so long as the capillaries are  $>20\ \mu\text{m}$  in lateral dimension.<sup>19</sup> The procedure we use—which we call “rapid prototyping”—consists of four steps: (i) high-resolution printing of a mask with  $>20\text{-}\mu\text{m}$  features; (ii) photolithography with this mask to fabricate a bas-relief master; (iii) micromolding against this master to form three sides of the channel system; and (iv) sealing to form the complete microchannel. It is straightforward to create multiple streams of fluids flowing laminarly in parallel inside these channels<sup>20</sup> and to use these streams for microfabrication and micropatterning by localized delivery of reagents from the laminar flows. This method—sometimes in combination with the use of prepatterned channels—allows fabrication of complex functional structures inside preformed capillaries without the registration steps required by planar photolithography.

Microfabrication by delivery of reactants from parallel laminar flows generates structures (etched or deposited) having edges tapered along their longitudinal direction as a result of diffusion transverse to the direction of flow.<sup>20,26</sup> This diffusional broadening can be minimized by using high flow rates ( $v \approx 0.50\ \text{m s}^{-1}$ ); high rates of flow result in short residence times in the region being patterned. The resolution one can achieve depends on the details of the process being used for patterning.

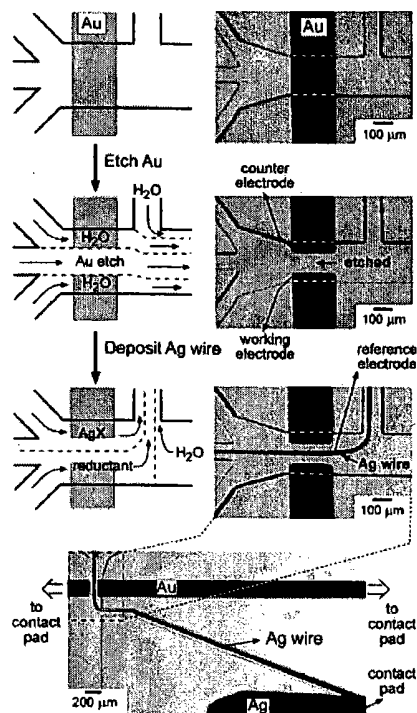
**Area-Selective Crystal Growth.** A two-stream laminar flow can be used for the localized nucleation and growth of crystals inside a microchannel.<sup>20</sup> The capillary is fabricated by placing a patterned PDMS membrane on a gold-coated wafer covered with a self-assembled monolayer (SAM) of  $\text{HS}(\text{CH}_2)_{15}\text{COOH}$ ; this SAM nucleates formation of calcite crystals.<sup>21</sup> Figure 4A shows a line of calcite crystals—with diameters of 2–5  $\mu\text{m}$ —that have nucleated and grown at the interface between two parallel streams, one containing calcium ions (as  $\text{CaCl}_2$ ) and the other containing carbonate ions (as  $\text{NaHCO}_3$ ).

**Patterned Etching of Silver and PDMS.** Figure 4B shows the result of patterning the inside of a glass capillary by electroless deposition of silver from one stream of a two-stream fluid flow system. The glass capillary is connected to a Y-shaped channel in PDMS in order to create the two-stream flow system; the method can be extended to four or more streams.<sup>22</sup> Figure 4C shows the creation of topographical features inside of a PDMS capillary using patterned flow of etchant and inert solvent.<sup>23</sup> In general, the position (or edges) of the etched or deposited micro-



**FIGURE 4.** (A) Scanning electron microscopy images of calcite deposited on a self-assembled monolayer at the interface of aqueous laminar flows of  $\text{NaHCO}_3$  (100 mM, buffered to pH 7.4) and  $\text{CaCl}_2$  (25 mM). (B) Optical micrograph of a glass capillary that has half of the inner surface plated with silver. (C) Optical micrograph of topographical features created inside of a PDMS capillary using patterned flow of NMP (*N*-methylpyrrolidinone, an inert liquid) and TBAF (tetrabutylammonium fluoride in NMP; an etchant). Inset Schematic cross section of the topographically patterned channel. structure can be controlled with a precision down to  $\sim 5 \mu\text{m}$  by adjustment of the relative volumes of the streams being injected.

**Three-Electrode System.** Figure 5 outlines a two-step fabrication of a three-electrode array inside a preformed channel. This procedure starts by placing a PDMS membrane with the "crow's foot" channel system embossed in its surface on a glass slide having a 200- $\mu\text{m}$ -wide evaporated gold stripe.<sup>20</sup> A three-phase laminar flow through the channel—a central gold-etchant solution, and adjacent water streams—selectively removes the gold in a strip in the middle of the channel. This etching creates two isolated Au electrodes that serve as working and counter electrodes. In a subsequent step, flowing the two components of a commercial electroless silver plating solution laminarily in parallel through the channel results in the deposition of a metallic silver wire on the glass at the interface between the two phases. Figure 5 also shows a typical example of tapering or broadening of a microstructure—here the silver wire—along the longitudinal direction of the channel as a result of diffusion transverse to the direction of flow. After treatment with 1% HCl to form AgCl on the surface, the silver wire serves as the reference electrode. We have shown that continuous, conductive silver wires as thin as  $10 \mu\text{m}$  can be fabricated by using high flow rates (up to  $1 \text{ m s}^{-1}$ ). For the fabrication of the silver wire in the three-electrode system, we typically used lower flow rates. The lower rates gave wider and less uniform wires, but these wires were also desirable (in this instance) for durability in use. We successfully recorded



**FIGURE 5.** Optical micrographs of the stepwise fabrication of a three-electrode system using multiphase laminar flow. A PDMS mold with a channel system embossed in its surface is placed on a glass slide covered with an evaporated gold strip. Two electrodes (counter and working) are formed by selectively etching the gold stripe in the middle of the channel with a three-phase laminar flow system. The gold stripe widens beyond the outer edge of the PDMS membrane into contact pads. A silver (reference) electrode is fabricated at the interface of a two-phase laminar flow and is directed into a side channel toward a contact pad.

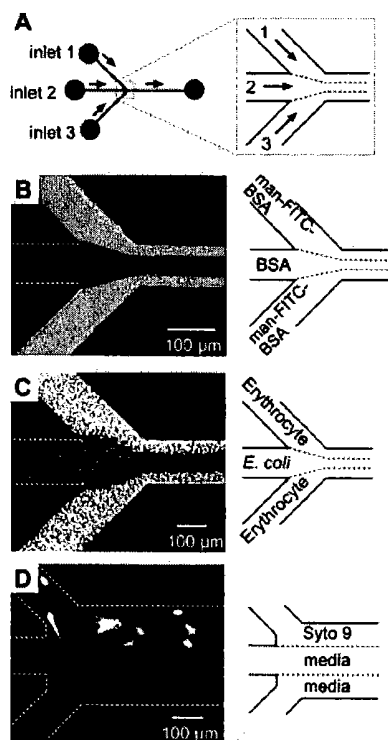
cyclic voltammograms of, for example, a 5-nL volume of 2 mM  $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$  in water.<sup>20</sup>

#### Patterning Proteins and Cells on Capillary Surfaces.

Multistream laminar flow systems are useful in fabricating biologically relevant structures and patterns in sizes similar to those of cells (typically 5–100  $\mu\text{m}$ ). Figure 6 shows three examples of patterning within a capillary: (A) proteins adsorbed on the substrate; (B) the location of cells; and (C) the delivery of biochemicals to cells.<sup>24</sup> These methods will be useful in fabricating microfluidic systems for cell-based sensors and screening systems based on cells and for studying chemotaxis, cell–cell communication, and cellular ecology.

## Conclusions

Microfabrication with liquids flowing in capillaries has characteristics that are fundamentally different from those of microfabrication with photolithography. The former relies on microfluidic channels to deliver reagents in patterns of flowing liquids to specific regions of a surface; the latter relies on masks to pattern light. Fabrication of the microchannels is a relatively straightforward task, using a rapid prototyping strategy based on soft lithography;<sup>19</sup> photolithography usually requires more specialized tools. Microfluidic systems are limited in the patterns



**FIGURE 6.** Examples of biologically relevant patterns generated using multiphase laminar flow. (A) Top view of a capillary network used for biological patterning. Optical micrographs B–D (fluorescence) represent close-up views of the junctions where the inlets converge into a single main channel. (B) Creating patterns of proteins inside a capillary. Solutions of bovine serum albumin (BSA) and BSA colabeled with mannose and fluorescein (man-FITC-BSA) were allowed to flow through the designated channels. (C) Patterned deposition of cells inside a capillary. Chick erythrocytes and *Escherichia coli* were deposited selectively in their designated lanes by patterned flow of cell suspensions. Adherent cells were visualized with a fluorescent nucleic acid stain (Syto 9). (D) Using patterned flow of media to stain part of the bovine capillary endothelial cells that were attached to the entire bottom face of a capillary. Only those cells over which a solution of dye (Syto 9) was allowed to flow were labeled. Syto 9 and media were allowed to flow from the designated inlets.

they can form; photolithography is a very general method of forming patterns.

The occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents. The width of the separate streams (or the interfacial width as governed by the rates of diffusion) internal to the channel determines the feature sizes, and the relative volumes of the streams control the position of the liquid–liquid interfaces. External positioning tools or masks determine feature sizes and positions in photolithography. The usual problem in microfabrication based on photolithography of determining position in superimposing patterns in multilayer fabrication (registration) translates into a problem of controlling relative rates of flow in microfluidic fabrication.

Microfluidic and photolithographic patterning are sufficiently different in the physical principles on which they

rest that they are complementary in their applications. Fluidic microfabrication inside capillaries is presently best suited for one-at-a-time, research, or prototyping demonstrations. Its strengths are convergence, specific applicability to problems involving channels (e.g., microanalytical devices), and compatibility with biological reagents and systems. Photolithography is highly developed for in-plane microfabrication and can be used to make many devices in parallel. Its strengths are generality in patterning, the ability to make small features, the ability to manufacture small systems in large numbers, and performance highly optimized for use with semiconductors and ceramics.

Fluid flow in microchannels allows the fabrication of a variety of useful structures ranging from conducting organic fibers to electrode systems integrated into microanalytical devices. Fluid flow also has the ability to deposit or carve away material on the interior surface of the capillary, and thus to change the surface properties or topography of a channel. Such patterns can have a profound effect on the flow profiles. For example, electroosmotic flow in capillaries that are patterned with regions of opposite surface charge leads to unusual low-*Re* flows.<sup>25</sup> Studies of the details of the fluid flow in microfluidic systems—by visualization or modeling—should make it possible to fabricate a broader range of structures using fluid flow.<sup>26</sup>

Fluid flows have clear potential to pattern delicate biological structures. They are also applicable to patterning the topography of the surfaces, the proteins absorbed on these surfaces, cells attached to those surfaces, and the medium to which the cells are exposed. We expect these methods to facilitate the study of, for example, cell–surface adhesion, chemotaxis, and cell–cell communication, and to contribute to cell-based microfluidic sensors.

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## References

- (1) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane). *Anal. Chem.* **1998**, *70*, 4974–4984.
- (2) Madou, M. *Fundamentals of Microfabrication*; CRC Press: New York, 1997.
- (3) Matsuura, Y.; Miura, D.; Miyagi, M. Fabrication of Copper Oxide-Coated Hollow Waveguides for CO<sub>2</sub> Laser Radiation. *Appl. Opt.* **1999**, *38*, 1700–1703.
- (4) Renn, M. J.; Pastel, R. Particle Manipulation and Surface Patterning by Laser Guidance. *J. Vac. Sci. Technol. B.* **1998**, *16*, 3859–3863.
- (5) The characteristics of fluid flow in capillaries have been studied extensively in the past. See, for example: Rosenhead, L. *Laminar Boundary Layers*; Dover Publications: New York, 1988. Tritton, D. J. *Physical Fluid Dynamics*, 2nd ed.; Oxford University Press: New York, 1988.

- (6) Fuh, C. B.; Levin, S.; Giddings, J. C. Rapid Diffusion Coefficient Measurements Using Analytical SPLITT Fractionation: Application to Proteins. *Anal. Biochem.* **1993**, *208*, 80–87. Levin, S.; Tawil, G. Analytical SPLITT Fractionation in the Diffusion Mode Operating as a Dialysis-like System Devoid of Membrane. Application to Drug-Carrying Liposomes. *Anal. Chem.* **1993**, *65*, 2254–2261.
- (7) Weigl, B. H.; Yager, P. Silicon Microfabricated Diffusion-Based Optical Chemical Sensor. *Sens. Actuators B* **1997**, *38–39*, 452–457. Weigl, B. H.; Yager, P. Microfluidic Diffusion based Separation and Detection. *Science* **1990**, *283*, 346–347.
- (8) Rodriguez, I.; Li, S. F. Y. Surface Deactivation in Protein and Peptide Analysis by Capillary Electrophoresis. *Anal. Chim. Acta* **1999**, *383*, 1–26. Altria, K. D. Overview of Capillary Electrophoresis and Capillary Electrochromatography. *J. Chromatogr. A* **1999**, *856*, 443–463.
- (9) Abe, Y.; Matsuura, Y.; Shi, Y.-W.; Wang, Y.; Uyama, H.; Miyagi, M. Polymer-Coated Hollow Fiber for CO<sub>2</sub> Laser Delivery. *Opt. Lett.* **1998**, *23*, 89–90.
- (10) Li, S.; Macosko, C. W.; White, H. S. Electrochemical Processing of Conducting Polymer Fibers. *Science* **1993**, *259*, 957–960. Li, S.; Macosko, C. W.; White, H. S. Electrochemical Processing of Electrically Conductive Polymer Fibers. *Adv. Mater.* **1993**, *5*, 575–576. Li, S.; White, H. S. Synthesis of Conducting Polymer Composite Fibers in Electrochemical Flow Cells. *J. Electrochem. Soc.* **1993**, *140*, 2473–2476.
- (11) Lambelet, P.; Sayah, A.; Pfeffer, M.; Philipona, C.; Marquis-Weible, F. Chemically Etched Fiber Tips for Near-Field Optical Microscopy: A Process for Smoother Tips. *Appl. Opt.* **1998**, *37*, 7289–7292.
- (12) Stöckle, R.; Fokas, C.; Deckert, V.; Zenobi, R.; Sick, B.; Hecht, B.; Wild, U. P. High-Quality Near-Field Optical Probes by Tube Etching. *Appl. Phys. Lett.* **1999**, *75*, 160–162.
- (13) Xia, Y.; Kim, E.; Whitesides, G. M. Micromolding in Capillaries: Applications in Materials Science. *J. Am. Chem. Soc.* **1996**, *118*, 5722–5731.
- (14) Delamarche, E.; Bernard, A.; Schmid, H.; Michel, B.; Biebuyck, H. Patterned Delivery of Immunoglobulins to Surfaces Using Microfluidic Networks. *Science* **1997**, *276*, 779–781. Delamarche, E.; Bernard, A.; Schmid, H.; Bietsch, A.; Michel, B.; Biebuyck, H. Microfluidic Networks for Chemical Patterning of Substrates: Design and Application to Bioassays. *J. Am. Chem. Soc.* **1998**, *120*, 500–508.
- (15) Ocvirk, G.; Verpoorte, E.; Manz, A.; Grasserbauer, M.; Widmer, H. M. High-Performance Liquid-Chromatography Partially Integrated onto a Silicon Chip. *Anal. Methods Instrum.* **1995**, *2*, 74–82. Oleschuk, R. D.; Schultz-Lockyear, L. L.; Ning, Y.; Harrison, D. J. Trapping of Bead-Based Reagents within Microfluidic Systems: On-Chip Solid-Phase Extraction and Electrochromatography. *Anal. Chem.* **2000**, *72*, 585–590.
- (16) Park, S. H.; Xia, Y. Macroporous Membranes with Highly Ordered and Three-Dimensionally Interconnected Spherical Pores. *Adv. Mater.* **1998**, *10*, 1045–1048. Park, S. H.; Xia, Y. Fabrication of Three-Dimensional, Macroporous Membranes with Assemblies of Microspheres as Templates. *Chem. Mater.* **1998**, *10*, 1745–1747.
- (17) Park, S. H.; Xia, Y. Assembly of Mesoscale Particles over Large Areas and Its Application in Fabricating Tunable Optical Filters. *Langmuir* **1999**, *15*, 266–273.
- (18) Park, S. H.; Gates, B.; Xia, Y. A Three-Dimensional Photonic Crystal Operating in the Visible Region. *Adv. Mater.* **1999**, *11*, 462–466.
- (19) Xia, Y.; Whitesides, G. M. Soft Lithography. *Angew. Chem., Int. Ed.* **1998**, *37*, 550–575.
- (20) Kenis, P. J. A.; Ismagilov, R. F.; Whitesides, G. M. Microfabrication Inside Capillaries Using Multiphase Laminar Flow Patterning. *Science* **1999**, *285*, 83–85.
- (21) Aizenberg, J.; Black, A. J.; Whitesides, G. M. Controlling Local Disorder in Self-Assembled Monolayers by Patterning the Topography of their Metallic Supports. *Nature* **1998**, *394*, 868–871. Aizenberg, J.; Black, A. J.; Whitesides, G. M. Control of Crystal Nucleation by Patterned Self-Assembled Monolayers. *Nature* **1999**, *398*, 495–498.
- (22) Ismagilov, R. F.; Kenis, P. J. A.; Stroock, A. D.; Rosmarin, D.; Whitesides, G. M., unpublished results.
- (23) Takayama, S.; Ostuni, E.; McDonald, J. C.; Bowden, N.; Wu, M.-H.; Whitesides, G. M., unpublished results.
- (24) Takayama, S.; McDonald, J. C.; Ostuni, E.; Liang, M.; Kenis, P. J. A.; Ismagilov, R. F.; Whitesides, G. M. Patterning Cells and their Environments Using Multiple Laminar Fluid Flows in Capillary Networks. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5545–5548.
- (25) Stroock, A. D.; Chiu, D. T.; Huck, W. T. S.; Ismagilov, R. F.; Kenis, P. J. A.; Weck, M.; Stone, H. A.; Whitesides, G. M. Patterning of Electro Osmotic Flow with Patterned Surface Charges. *Phys. Rev. Lett.* **2000**, *84*, 3314–3317.
- (26) Ismagilov, R. F.; Stroock, A. D.; Kenis, P. J. A.; Stone, H. A.; Whitesides, G. M. Experimental and Theoretical Scaling Laws for Transverse Diffusive Broadening in Two-Phase Laminar Flows in Microchannels. *Appl. Phys. Lett.* **2000**, *76*, 2376–2378. General reference: Merzkirch, W. *Flow Visualization*, 2nd ed.; Academic Press: Orlando, FL, 1987.

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